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Novel Betaherpesvirus in Bats

Technical Appendix 1

System for Rapid Determination of Viral RNA Sequences, Version 3.1

The system for rapid determination of viral RNA sequences (RDV) version 3.0 (*1*) was modified to simplify the procedure. The modified method was designated version 3.1. Adapters and primers for construction of second cDNA library in RDV version 3.1 were newly designed and used instead of those in RDV version 3.0 (see Figure below. A: adaptors, B: primers). Both adapters have sticky-end structures digested with *Sau*3AI or *Hpy*CH4IV. The RDV version 3.1 method includes the 4 procedures described below.

1. RNA extraction. To eliminate contaminating cellular RNA and DNA from the samples, culture supernatant was treated with DNase I and RNase A as described previously (*2*), following RNA extraction by using a Total RNA isolation mini kit (Agilent Technologies, Santa Clara, CA, USA).
2. Construction of first cDNA library. A whole-transcriptome amplification system (WTA; Sigma-Aldrich, Saint Louis, MO, USA) was used to amplify double-stranded viral cDNA as described previously (*3*).
3. Construction of second cDNA library. After the first cDNA library purification with the MonoFas DNA isolation system (GL Sciences, Tokyo, Japan), DNA was

digested with 20 U of *Hpy*CH4IV (New England Biolabs, Ipswich, MA, USA) and *Sau*3AI (New England Biolabs) at 37°C for 60 min, and the digested DNA was again purified by using MonoFas. For construction of the second cDNA library, aliquots of 2.5 uL of DNA solution, 2.5 uL of distilled water, 2.5 uL of RDV-Adaptor-*Sau*3AI adaptor (10 uM) and RDV-Adaptor-*Hpy*CH4IV adaptor (10 uM) were mixed. The sequences and structures of the adapters are shown in figure below. A ligation-convenience kit (Nippon Gene, Toyama, Japan) was used for adaptor ligation. The DNA solution and 10 uL of ligation mix were reacted at 16°C for 30 min, and the DNA was isolated by using MonoFas. The second cDNA library was amplified by PCR with specially designed primer sets; the forward primers had 5 nt including GATC (*Sau*3AI-digested sequence) and 1 variable nucleotide added to the 3′ end of the RDV-Adaptor-*Sau*3AI sequence, and the reverse primers had 6 nt including ACGT (*Hpy*CH4IV-digested sequence) and 2 variable nucleotides added to the 3′ end of the RDV-Adaptor-*Hpy*CH4IV sequence. The PCR mixture was prepared by mixing 15 uL of AmpliTaq Gold PCR Master Mix containing AmpliTaq Gold, 0.5 uL of forward primer for RDV-Adaptor-*Sau*3AI (10 uM), 0.5 uL of reverse primer for RDV-Adaptor-*Hpy*CH4IV (10 uM), 1 uL of DNA solution, and 13 uL of distilled water. PCR was performed by using 64 primers under the conditions described previously (*1*).

4. Direct sequencing. After electrophoresis of PCR products on agarose gels, bands >120 bp were excised, and DNA was extracted from the gel by using MonoFas. Direct sequencing was performed by using the forward or reverse primer.

References

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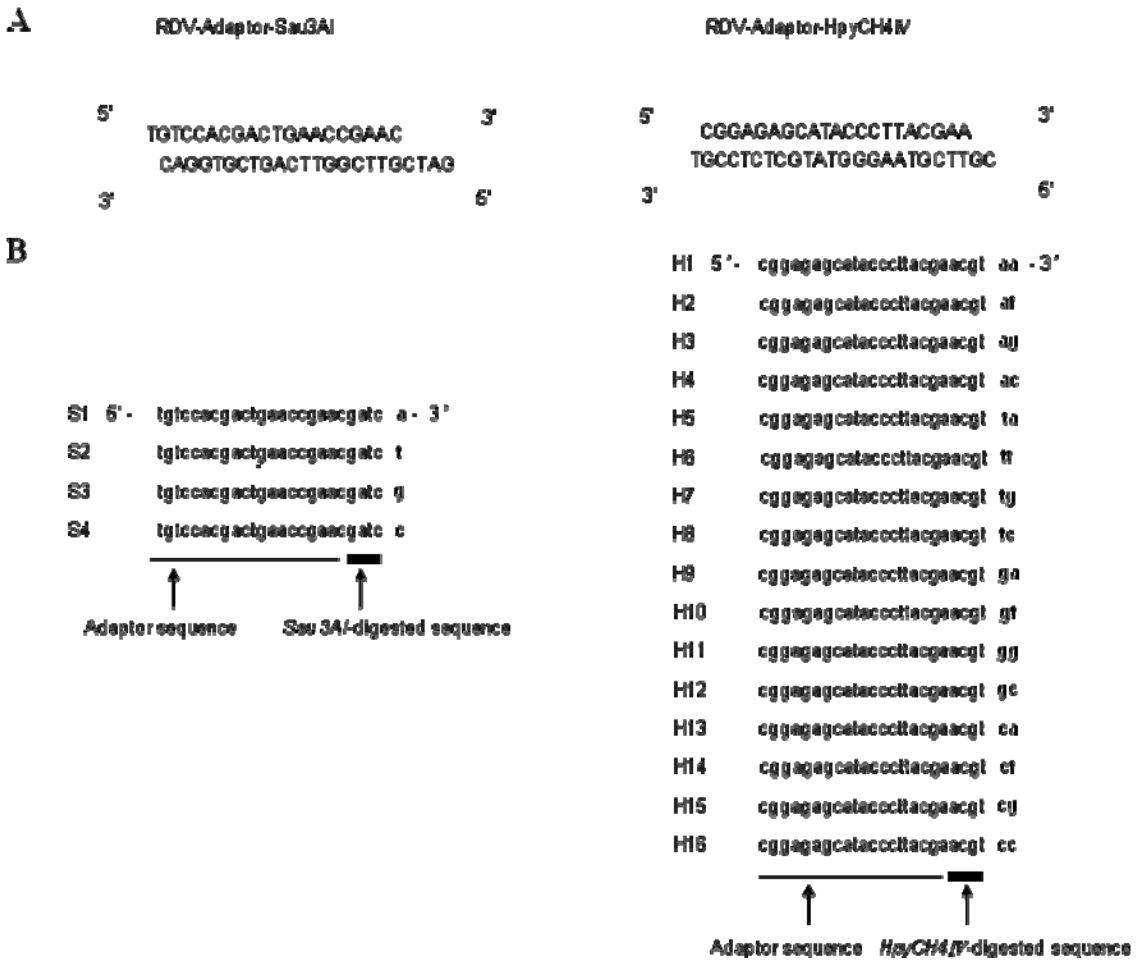
A
RDV-Adaptor-Sau3AI
5'
TGTCCACGACTGAACCGAAC
3'
CAGGTGCTGACTTGGCTTGCTAG
3'
6'
RDV-Adaptor-HpyCH4IV
5'
CGGAGAGCATACCCTTACGAA
3'
TGCCTCTCGTATGGGAATGCTTGC
3'
6'
B
S1 6'- tgtccacgactgaaccgaacgatc a - 3'
S2 tgtccacgactgaaccgaacgatc t
S3 tgtccacgactgaaccgaacgatc g
S4 tgtccacgactgaaccgaacgatc c
Adaptor sequence
Sau 3AI-digested sequence
H1 5'- cggagagcataccottacgaacgt aa - 3'
H2 cggagagcataccottacgaacgt at
H3 cggagagcataccottacgaacgt ag
H4 cggagagcataccottacgaacgt ac
H5 cggagagcataccottacgaacgt ta
H6 cggagagcataccottacgaacgt tt
H7 cggagagcataccottacgaacgt tg
H8 cggagagcataccottacgaacgt tc
H9 cggagagcataccottacgaacgt ga
H10 cggagagcataccottacgaacgt gt
H11 cggagagcataccottacgaacgt gg
H12 cggagagcataccottacgaacgt gc
H13 cggagagcataccottacgaacgt ca
H14 cggagagcataccottacgaacgt ct
H15 cggagagcataccottacgaacgt cg
H16 cggagagcataccottacgaacgt cc
Adaptor sequence
HpyCH4IV-digested sequence